L Number	Hits	Search Text	DB	Time stamp
1	16	primer adj1 extension near5 (different or plurality adj2 primers and tag)	USPAT; US-PGPUB; DERWENT	2003/01/30 10:30
2	150	primer adj1 extension near5 (different or plurality adj2 primers)	USPAT; US-PGPUB; DERWENT	2003/01/30 10:31
3	3	(primer adj1 extension near5 (different or plurality adj2 primers)) and repeat adj2 unit	USPAT; US-PGPUB; DERWENT	2003/01/30 10:31

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 NEWS  3  Apr 09   BEILSTEIN: Reload and Implementation of a New Subject Area
 NEWS  4  Apr 09   ZDB will be removed from STN
 NEWS  5  Apr 19   US Patent Applications available in IFICDB, IFIPAT, and
IFIUDB
 NEWS  6  Apr 22   Records from IP.com available in CAPLUS, HCAPLUS, and
ZCAPLUS
 NEWS  7  Apr 22   BIOSIS Gene Names now available in TOXCENTER
 NEWS  8  Apr 22   Federal Research in Progress (FEDRIP) now available
 NEWS  9  Jun 03   New e-mail delivery for search results now available
 NEWS 10  Jun 10   MEDLINE Reload
 NEWS 11  Jun 10   PCTFULL has been reloaded
 NEWS 12  Jul 02   FOREGE no longer contains STANDARDS file segment
 NEWS 13  Jul 22   USAN to be reloaded July 28, 2002;
                   saved answer sets no longer valid
 NEWS 14  Jul 29   Enhanced polymer searching in REGISTRY
 NEWS 15  Jul 30   NETFIRST to be removed from STN
 NEWS 16  Aug 08   CANCERLIT reload
 NEWS 17  Aug 08   PHARMAMarketLetter(PHARMAML) - new on STN
 NEWS 18  Aug 08   NTIS has been reloaded and enhanced
 NEWS 19  Aug 19   Aquatic Toxicity Information Retrieval (AQUIRE)
                   now available on STN
 NEWS 20  Aug 19   IFIPAT, IFICDB, and IFIUDB have been reloaded
 NEWS 21  Aug 19   The MEDLINE file segment of TOXCENTER has been reloaded
 NEWS 22  Aug 26   Sequence searching in REGISTRY enhanced
 NEWS 23  Sep 03   JAPIO has been reloaded and enhanced
 NEWS 24  Sep 16   Experimental properties added to the REGISTRY file
 NEWS 25  Sep 16   CA Section Thesaurus available in CAPLUS and CA
 NEWS 26  Oct 01   CASREACT Enriched with Reactions from 1907 to 1985
 NEWS 27  Oct 21   EVENTLINE has been reloaded
 NEWS 28  Oct 24   BEILSTEIN adds new search fields
 NEWS 29  Oct 24   Nutraceuticals International (NUTRACEUT) now available on
STN
 NEWS 30  Oct 25   MEDLINE SDI run of October 8, 2002
 NEWS 31  Nov 18   DKILIT has been renamed APOLLIT
 NEWS 32  Nov 25   More calculated properties added to REGISTRY
 NEWS 33  Dec 02   TIBKAT will be removed from STN
 NEWS 34  Dec 04   CSA files on STN
 NEWS 35  Dec 17   PCTFULL now covers WP/PCT Applications from 1978 to date
 NEWS 36  Dec 17   TOXCENTER enhanced with additional content
 NEWS 37  Dec 17   Adis Clinical Trials Insight now available on STN
 NEWS 38  Dec 30   ISMEC no longer available
 NEWS 39  Jan 13   Indexing added to some pre-1967 records in CA/CAPLUS
 NEWS 40  Jan 21   NUTRACEUT offering one free connect hour in February 2003
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NEWS 41 Jan 21 PHARMAML offering one free connect hour in February 2003
NEWS 42 Jan 29 Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC

NEWS EXPRESS January 6 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
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FILE 'HOME' ENTERED AT 11:09:43 ON 30 JAN 2003

=> FIL BIOSIS MEDLINE EMBASE LIFESCI CAPLUS

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FILE 'BIOSIS' ENTERED AT 11:09:58 ON 30 JAN 2003
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=> primer (2a) extension (P) sequence(2a) tag (s) primers
PRIMER IS NOT A RECOGNIZED COMMAND
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For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s primer (2a) extension (P) sequence(2a) tag (s) primers
L1 5 PRIMER (2A) EXTENSION (P) SEQUENCE(2A) TAG (S) PRIMERS

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=> dup rem l1
PROCESSING COMPLETED FOR L1
L2             5 DUP REM L1 (0 DUPLICATES REMOVED)

=> d l1 1-5

L1   ANSWER 1 OF 5  CAPLUS  COPYRIGHT 2003 ACS
AN   2002:638197  CAPLUS
DN   137:180749
TI   Detection of genetic polymorphisms using generic molecular beacon probes
     labeled with fluoresce dye-conjugated metallic or semiconductor
     nanoparticles
IN   Phillips, Vince; Watson, Andrew R.; Wong, Edith
PA   USA
SO   U.S. Pat. Appl. Publ., 27 pp.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.         KIND  DATE          APPLICATION NO.  DATE
     ---------------    ----  --------      ---------------  --------
PI   US 2002115082      A1    20020822      US 2001-945379   20010831
PRAI US 2000-230186P    P     20000901

L1   ANSWER 2 OF 5  CAPLUS  COPYRIGHT 2003 ACS
AN   2001:816979  CAPLUS
DN   135:353731
TI   Methods and compositions for polynucleotide analysis using generic
capture
     sequences
IN   Lai, Jennifer H.; Phillips, Vince E.; Watson, Andrew R.
PA   Quantum Dot Corporation, USA
SO   PCT Int. Appl., 85 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.         KIND  DATE          APPLICATION NO.  DATE
     ---------------    ----  --------      ---------------  --------
PI   WO 2001083823      A1    20011108      WO 2001-US13979  20010430
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
             HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
             LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
             RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ,
             VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
             BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRAI US 2000-200635P    P     20000428
RE.CNT  3      THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
               ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1   ANSWER 3 OF 5  CAPLUS  COPYRIGHT 2003 ACS
AN   2001:300909  CAPLUS
DN   134:321563
TI   Allele detection using primer extension with sequence-coded identity tags
IN   Huang, Xiaohua; Ryder, Tom; Kaplan, Paul
PA   Affymetrix, Inc., USA
SO   PCT Int. Appl., 42 pp.
```

CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001029260	A2	20010426	WO 2000-US28284	20001013
	WO 2001029260	A3	20020117		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6287778	B1	20010911	US 1999-420805	19991019
	US 2002025532	A1	20020228	US 2001-948756	20010910
PRAI	US 1999-420805	A1	19991019		

L1 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS
AN 2000:790664 CAPLUS
DN 133:345537
TI Amplification of nucleic acids using interlaced nesting primers for use in large-scale sequencing projects
IN Tillett, Daniel
PA Takara Shuzo Co., Ltd., Japan
SO PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000066768	A1	20001109	WO 2000-AU391	20000501
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	EP 1181389	A1	20020227	EP 2000-918612	20000501
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002542837	T2	20021217	JP 2000-615790	20000501
PRAI	AU 1999-87	A	19990430		
	WO 2000-AU391	W	20000501		

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS
AN 1999:633269 CAPLUS
DN 131:267933
TI A method for sequencing very long DNAs with a small set of primers that can be mutated and adapted to novel sequence information

IN Brenner, Sydney
PA Lynx Therapeutics, Inc., USA
SO U.S., 28 pp., Cont.-in-part of U.S. 5,780,231.
    CODEN: USXXAM
DT Patent
LA English
FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5962228	A	19991005	US 1997-916120	19970822
	US 5763175	A	19980609	US 1995-560313	19951117
	US 5780231	A	19980714	US 1996-611155	19960305
	JP 11151092	A2	19990608	JP 1998-237840	19980824
PRAI	US 1995-560313		19951117		
	US 1996-611155		19960305		
	US 1997-916120		19970822		

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
          ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s primer (2a) extension and and hybridiz?
MISSING TERM 'AND AND'
The search profile that was entered contains a logical
operator followed immediately by another operator.

=> s primer (2a) extension and hybridiz?
L3 4062 PRIMER (2A) EXTENSION AND HYBRIDIZ?

=> s l3 and array
L4 114 L3 AND ARRAY

=> s l4 and different
L5 23 L4 AND DIFFERENT

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 12 DUP REM L5 (11 DUPLICATES REMOVED)

=> d l6 1-12

L6 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE
    1
AN 2002:308639 BIOSIS
DN PREV200200308639
TI Methods for gene expression monitoring on electronic microarrays.
AU Weidenhammer, Elaine M. (1); Wang, Ling; Xu, Xiao; Heller, Michael J.;
    Kahl, Brenda F.
CS (1) San diego, CA USA
    ASSIGNEE: Nanogen, Inc.
PI US 6379897 April 30, 2002
SO Official Gazette of the United States Patent and Trademark Office
Patents,
    (Apr. 30, 2002) Vol. 1257, No. 5, pp. No Pagination.
    http://www.uspto.gov/web/menu/patdata.html. e-file.
    ISSN: 0098-1133.
DT Patent
LA English

L6 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2002:946900 CAPLUS

DN 138:20450
TI Fluorescent microsphere-based readout technology for multiplexed human single nucleotide polymorphism analysis and bacterial identification
IN Casey, Warren Michael; Chen, Jingwen; Colton, Heidi M.; Taylor, David; Weiner, Michael Phillip
PA USA
SO U.S. Pat. Appl. Publ., 58 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002187470	A1	20021212	US 2000-745317	20001221
PRAI	US 2000-745317		20001221		

L6 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2002:632737 CAPLUS
DN 137:180735
TI Solid phase sequencing of double-stranded nucleic acids by **array hybridization** and mass spectrometry
IN Fu, Dong-Jing; Cantor, Charles R.; Koster, Hubert; Smith, Cassandra L.
PA Boston University, USA; Sequenom, Inc.
SO U.S., 79 pp., Cont.-in-part of U.S. Ser. No. 420,009, abandoned.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 18

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6436635	B1	20020820	US 1996-614151	19960312
	US 5795714	A	19980818	US 1993-110691	19930823
	EP 1262564	A2	20021204	EP 2002-16384	19940106
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
	US 5631134	A	19970520	US 1995-462704	19950605
	CA 2218188	AA	19961017	CA 1996-2218188	19960410
	WO 9632504	A2	19961017	WO 1996-US5136	19960410
	WO 9632504	A3	19961114		
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML				
	AU 9655446	A1	19961030	AU 1996-55446	19960410
	EP 830460	A1	19980325	EP 1996-912743	19960410
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
	JP 11503611	T2	19990330	JP 1996-531243	19960410
	AU 9891379	A1	19990114	AU 1998-91379	19981106
	AU 738203	B2	20010913		
PRAI	US 1992-972012	B2	19921106		
	US 1993-1323	B2	19930107		
	US 1993-110691	A2	19930823		
	US 1995-419994	B2	19950411		
	US 1995-420009	B2	19950411		
	AU 1994-59929	A3	19940106		
	EP 1994-906047	A3	19940106		
	US 1994-322526	A3	19941017		

US 1996-614151 A 19960312
WO 1996-US5136 W 19960410
RE.CNT 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 12 MEDLINE
AN 2002716929 MEDLINE
DN 22366777 PubMed ID: 12479262
TI Characterization of overlapping XAGE-1 transcripts encoding a cancer testis antigen expressed in lung, breast, and other types of cancers.
AU Egland Kristi A; Kumar Vasantha; Duray Paul; Pastan Ira
CS Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda,
Maryland 20892-4264, USA.
SO Mol Cancer Ther, (2002 May) 1 (7) 441-50.
Journal code: 101132535. ISSN: 1535-7163.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200301
ED Entered STN: 20021218
Last Updated on STN: 20030114
Entered Medline: 20030113

L6 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2001:935817 CAPLUS
DN 136:65168
TI Method for detecting cytosine methylation
IN Berlin, Kurt
PA Epigenomics A.-G., Germany
SO PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001098528	A2	20011227	WO 2001-DE2274	20010619
	WO 2001098528	A3	20021128		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	DE 10029915	A1	20020103	DE 2000-10029915	20000619
PRAI	DE 2000-10029915	A	20000619		

L6 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2001:300909 CAPLUS
DN 134:321563
TI Allele detection using **primer extension** with sequence-coded identity tags
IN Huang, Xiaohua; Ryder, Tom; Kaplan, Paul
PA Affymetrix, Inc., USA
SO PCT Int. Appl., 42 pp.

CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001029260	A2	20010426	WO 2000-US28284	20001013
	WO 2001029260	A3	20020117		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
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	US 6287778	B1	20010911	US 1999-420805	19991019
	US 2002025532	A1	20020228	US 2001-948756	20010910
PRAI	US 1999-420805	A1	19991019		

L6 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:180125 BIOSIS
DN PREV200100180125
TI High-throughput methods for detection of genetic variation.
AU Kristensen, Vessela Nedelcheva (1); Kelefiotis, Dimitris; Kristensen, Tom;
Borresen-Dale, Anne-Lise
CS (1) Institute for Cancer Research, Norwegian Radium Hospital, Montebello, 0310, Oslo: nedelcheva.vessela@dnr.uio.no Norway
SO Biotechniques, (February, 2001) Vol. 30, No. 2, pp. 318-332. print.
ISSN: 0736-6205.
DT Article
LA English
SL English

L6 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2001:214631 BIOSIS
DN PREV200100214631
TI Fluorescent microsphere-based readout technology for multiplexed human single nucleotide polymorphism analysis and bacterial identification.
AU Ye, Fei (1); Li, May-Sung; Taylor, J. David; Nguyen, Quan; Colton, Heidi M.; Casey, Warren M.; Wagner, Michael; Weiner, Michael P.; Chen, Jingwen
CS (1) Department of Genomic Sciences, GlaxoWellcome Research and Development, 5 Moore Drive, Research Triangle Park, NC, 27709-3398 USA
SO Human Mutation, (2001) Vol. 17, No. 4, pp. 305-316. print.
ISSN: 1059-7794.
DT Article
LA English
SL English

L6 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2001:267072 BIOSIS
DN PREV200100267072
TI Integration of multiple PCR amplifications and DNA mutation analyses by using oligonucleotide microchip.
AU Tillib, Sergei V.; Strizhkov, Boris N.; Mirzabekov, Andrei D. (1)

CS (1) Biochip Technology Center, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL, 60439: amir@anl.gov USA
SO Analytical Biochemistry, (May 1, 2001) Vol. 292, No. 1, pp. 155-160. print.
ISSN: 0003-2697.
DT Article
LA English
SL English

L6 ANSWER 10 OF 12 MEDLINE
AN 2000252328 MEDLINE
DN 20252328 PubMed ID: 10794354
TI Arrayed **primer extension**: solid-phase four-color DNA resequencing and mutation detection technology.
AU Kurg A; Tonisson N; Georgiou I; Shumaker J; Tollett J; Metspalu A
CS Institute of Molecular and Cell Biology, Tartu Children's Hospital, University of Tartu, Estonian Biocentre.
SO GENETIC TESTING, (2000) 4 (1) 1-7.
Journal code: 9802546. ISSN: 1090-6576.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000706
Last Updated on STN: 20000706
Entered Medline: 20000626

L6 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 1999:495415 CAPLUS
DN 131:140461
TI A method combining features of random amplified polymorphic DNA and arrayed **primer extension** for nucleic acid analysis
IN Ulfendahl, Per Johan
PA Amersham Pharmacia Biotech AB, Swed.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9939001	A2	19990805	WO 1999-EP918	19990202
	WO 9939001	A3	19991007		
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	EP 1051523	A2	20001115	EP 1999-911663	19990202
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	JP 2002501760	T2	20020122	JP 2000-529458	19990202
	US 6280954	B1	20010828	US 2000-601258	20000919
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	WO 1999-EP918	W	19990202		

L6 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 1998:323255 CAPLUS
DN 129:1411
TI High density immobilization of nucleic acids and apparatus for dispensing nanovolumes of liquids and formation of multielement **arrays**
IN O'Donnell, Maryanne J.; Cantor, Charles R.; Little, Daniel P.; Koster, Hubert
PA Sequenom, Inc., USA; O'Donnell, Maryanne J.; Cantor, Charles R.; Little, Daniel P.; Koster, Hubert
SO PCT Int. Appl., 157 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 18

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9820020	A2	19980514	WO 1997-US20195	19971106
	WO 9820020	A3	19981022		
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Executing the logoff script...

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	47.95	48.16

STN INTERNATIONAL LOGOFF AT 11:20:20 ON 30 JAN 2003

diagnosing hereditary non-polyposis colorectal cancer if said assay is positive and said colonoscopy reveals an adenoma,

wherein said nucleic acid mutation is a nucleic acid insertion or deletion, and

wherein said assay comprises the steps of:

(a) selecting a nucleic acid having a known wild-type sequence and having a target region comprising a repeat sequence having at most three different types of nucleotide bases selected from the group consisting of dGTP, dATP, dTTP, and dCTP;

(b) contacting a sample with an oligonucleotide primer that is complementary to a portion of said nucleic acid immediately upstream of said target region;

(c) extending said primer in the presence of nucleotide bases that are complementary to the nucleotide bases of the target region, thereby to form a primer extension product;

(d) extending the primer extension product in the presence of a labeled nucleotide complementary to a nucleotide base downstream from the target region in said nucleic acid, wherein said labeled nucleotide is not complementary to any of the nucleotide bases of the target region selected in step (a), thereby to produce a labeled extension product comprising a sequence that is complementary to the entire target region;

(e) terminating the primer extension product by incorporating a terminator nucleotide in said product that is complementary to a nucleotide downstream from the target region in a wild type nucleic acid, wherein said terminator nucleotide is not complementary to any of the nucleotides of the target region selected in step (a), said step of terminating the primer extension product being performed simultaneously with or immediately after step (d).

(f) detecting the labeled extension product; and

(g) comparing the size of the labeled extension product detected in step (f) to a standard, wherein a labeled extension product smaller than the standard is indicative of the presence of a deletion in the target region and a labeled extension product larger than the standard is indicative of the presence of an insertion in the target region.

8. The method of claim **1**, wherein the labeled nucleotide and the terminator nucleotide are the same.

9. The method of claim **1**, wherein more than one labeled nucleotide is incorporated into the extension product prior to incorporation of the terminator nucleotide.

10. The method of claim **1**, wherein the nucleotide bases of step (c) are unlabeled.

11. The method of claim **1**, wherein the labeling reaction of step (d) is performed in the presence of labeled nucleotide and unlabeled nucleotide of the same type.

12. The method of claim **10**, wherein the ratio of labeled nucleotides to unlabeled nucleotides is 1:1.6 (unlabeled:labeled).

13. The method of claim **10**, wherein more than one labeled nucleotide from step (d) is incorporated into the labeled extension product.

14. The method of claim **12**, wherein only one of the incorporated nucleotides is labeled.

15. The method of claim **1**, wherein said biological sample contains a heterogeneous mixture of mutant nucleic acid having a deletion in the target region and wild type nucleic acid with no deletion in the target region.

16. The method of claim **1**, wherein a deletion in the target region is present in from about 1% to about 5% of the nucleic acid molecules containing the target region.

17. The method of claim **1**, wherein the presence of a deletion in said target region is associated with the presence of a mutation at a separate genetic locus selected from the group consisting of APC, DCC, P53, and RAS.

18. The method of claim **1**, wherein said target region is the poly-A tract at the BAT-26 locus.

19. The method of claim **1**, wherein said target region is a microsatellite region.

* * * * *

-continued

gttaannnnn nnngacagat agtgaagaag gcttagaaag gagctaaaag agttcgacat 300

caatattaga caag 314

What is claimed is:

1. A method for diagnosing colorectal cancer or precancer, the method comprising the steps of:

performing an assay to detect, in a stool sample from a patient, a nucleic acid mutation indicative of a colorectal lesion;

performing a sigmoidoscopy on said patient; and

diagnosing colorectal cancer or precancer in said patient if at least one of said assay step and said sigmoidoscopy step is positive,

wherein said nucleic acid mutation is a nucleic acid insertion or deletion, and

wherein said assay comprises the steps of:

(a) selecting a nucleic acid having a known wild-type sequence and having a target region comprising a repeat sequence having at most three different types of nucleotide bases selected from the group consisting of dGTP, dATP, dTTP, and dCTP;

(b) contacting a sample with an oligonucleotide primer that is complementary to a portion of said nucleic acid immediately upstream of said target region;

(c) extending said primer in the presence of nucleotide bases that are complementary to the nucleotide bases of the target region, thereby to form a primer extension product;

(d) extending the primer extension product in the presence of a labeled nucleotide complementary to a nucleotide base downstream from the target region in said nucleic acid, wherein said labeled nucleotide is not complementary to any of the nucleotide bases of the target region selected in step (a), thereby to produce a labeled extension product comprising a sequence that is complementary to the entire target region;

(e) terminating the primer extension product by incorporating a terminator nucleotide in said product that is complementary to a nucleotide downstream from the target region in a wild type nucleic acid, wherein said terminator nucleotide is not complementary to any of the nucleotides of the target region selected in step (a), said step of terminating the primer extension product being performed simultaneously with or immediately after step (d);

(f) detecting the labeled extension product; and

(g) comparing the size of the labeled extension product detected in step (f) to a standard, wherein a labeled extension product smaller than the standard is indicative of the presence of a deletion in the target region and a labeled extension product larger than the standard is indicative of the presence of an insertion in the target region.

2. The method of claim 1, wherein said assay is conducted prior to said sigmoidoscopy.

3. The method of claim 1, wherein said sigmoidoscopy is performed prior to said assay.

4. The method of claim 1, wherein said mutation is indicative of the presence of a colorectal lesion in the proximal colon.

5. The method of claim 1, wherein said sample includes a buffer comprising at least 100 mM EDTA.

6. A method for localizing a colorectal lesion in a patient, the method comprising the steps of:

performing an assay to detect, in a stool sample from a patient, a nucleic acid mutation indicative of said colorectal lesion;

performing a sigmoidoscopy on said patient;

diagnosing a proximal colonic lesion if said assay is positive for the mutation and said sigmoidoscopy is negative; and

diagnosing a distal colonic lesion if said sigmoidoscopy is positive and said assay is negative for the mutation,

wherein said nucleic acid mutation is a nucleic acid insertion or deletion, and

wherein said assay comprises the steps of:

(a) selecting a nucleic acid having a known wild-type sequence and having a target region comprising a repeat sequence having at most three different types of nucleotide bases selected from the group consisting of dGTP, dATP, dTTP, and dCTP;

(b) contacting a sample with an oligonucleotide primer that is complementary to a portion of said nucleic acid immediately upstream of said target region;

(c) extending said primer in the presence of nucleotide bases that are complementary to the nucleotide bases of the target region, thereby to form a primer extension product;

(d) extending the primer extension product in the presence of a labeled nucleotide complementary to a nucleotide base downstream from the target region in said nucleic acid, wherein said labeled nucleotide is not complementary to any of the nucleotide bases of the target region selected in step (a), thereby to produce a labeled extension product comprising a sequence that is complementary to the entire target region;

(e) terminating the primer extension product by incorporating a terminator nucleotide in said product that is complementary to a nucleotide downstream from the target region in a wild type nucleic acid, wherein said terminator nucleotide is not complementary to any of the nucleotides of the target region selected in step (a), said step of terminating the primer extension product being performed simultaneously with or immediately after step (d).

(f) detecting the labeled extension product; and

(g) comparing the size of the labeled extension product detected in step (f) to a standard, wherein a labeled extension product smaller than the standard is indicative of the presence of a deletion in the target region and a labeled extension product larger than the standard is indicative of the presence of an insertion in the target region.

7. A method for diagnosing hereditary non-polyposis colorectal cancer, the method comprising the steps of:

performing an assay to detect, in a stool sample from a patient, a nucleic acid mutation indicative of said hereditary non-polyposis colorectal cancer;

performing a colonoscopy on said patient; and

centrifugation, dried, and dissolved in 20 μl mM Tris-HCl, pH 8.0; 1 mM EDTA.

Two microliters of each sample were electrophoresed on a 1.4% agarose gel (8.3-cm × 6 cm) in TAE buffer (40 mM Tris-acetate, pH 7.8; 5 mM sodium acetate: 1 mM ESTA) at 50 V for 160 min. After staining with ethidium, the gel was photographed using ultraviolet illumination. The results in FIG. 6 show that samples Hu1 and Hu2 have a prominent band at approximately 1.3 kb that is not present in the four other samples. Thus, amplification using the 10 nucleotide primer AP9 was able to detect a genetic polymorphism that can be used to distinguish human DNA samples.

EXAMPLE 8

Quantitation of PCR Products

In Example 3, the process of this invention was used to genetically map a polymorphism. This was accomplished by determining the parental pattern of inheritance of the polymorphism in several segregating individuals. As practiced in Example 3, the investigator is unable to distinguish individuals heterozygous for a marker from individuals homozygous for the same marker, and the polymorphism is scored as being dominant. A heterozygous individual contains two different copies (alleles) of a particular DNA segment at a position (locus) in the genome. A homozygous individual will contain two identical alleles at a locus. In several applications (e.g., quantitative trait mapping) it is necessary to be able to distinguish heterozygous individuals. In the process of the present invention, it is expected that individuals inheriting two copies of a single allele will have two times the amount of amplified product as an individual inheriting only one copy of the same allele. To investigate whether heterozygotes could be distinguished from homozygotes by quantitating the results of the assay, individuals were chosen which were known to be homozygous for Bonus, heterozygous for both Bonus and PI81762 or homozygous for PI81762 DNA segments for the chromosomal region containing the band A polymorphism. These individuals were chosen on the basis of the genotype of flanking RFLP markers (see Example 3 and Table 3). The same gels presented in Example 3 were analyzed by densitometry to determine the amount of amplified product corresponding to band A (see legend to FIG. 7). Panel A of FIG. 7 represents a scan of individual 30 (see Example 3) homozygous for the PI81762 allele. Panel B of FIG. 7 represents a scan of individual 9 (see Example 3) heterozygous for Bonus and PI81762 alleles. Panel C of FIG. 7 represents a scan of individual 57 (see Example 3) homozygous for the Bonus allele. Peaks corresponding to the band A polymorphism are labeled. The densitometry shows that individuals homozygous for PI81762 alleles contain twice the amount of DNA in the band A polymorphism than individuals which are heterozygous for PI81762 and Bonus. Individuals homozygous for the Bonus alleles do not contain any DNA corresponding to band A. This example shows that the products of amplification can be quantitated to identify heterozygous individuals and reveal a co-dominant polymorphism. This will have utility in procedures where it is necessary to distinguish heterozygotes from the corresponding homozygote.

Photographs of the gel separations shown in FIG. 2 were digitized using a Cohu Monochrome CCD camera (model #4815-5000, Cohu Inc., San Diego, Calif.) attached to a Macintosh IIcx computer (Apple Computer Corp., Cupertino, Calif.). The analog data was converted to digital input through a QuickCapture card (Data Translations Inc., Marlboro, Mass.). The individual lanes were scanned for quantitation with the Scan Analysis program (Version 2.11, Biosoft Inc., Milltown, N.J.). X-axis values correspond to electrophoretic distance, and Y-axis values correspond to peak intensity.

What is claimed is:

1. A process for detecting polymorphisms on the basis of nucleotide differences in random segments of nucleic acids comprising:
   (a) separately performing an extension reaction on a random segment of each of at least two nucleic acids from different sources, said reaction comprising:
      (i) contacting each of the nucleic acids with at least one nonspecifically targeted oligonucleotide primer of greater than 7 nucleotides under conditions such that for at least one nucleic acid a random extension product of at least one primer is synthesized; and
   (b) comparing the results of the separately performed random extension reactions for differences.

2. The process of claim 1, after step (i) and before step (b), further comprising the additional steps of (ii) dissociating the extension product from its complement; and (iii) amplifying the random nucleic acid segment by contacting the dissociated extension product with at least one primer of step (i) under conditions such that an amplification extension product is synthesized using the dissociated extension product as a template.

3. The process of claim 2 wherein the steps are performed at least twice.

4. The process of claim 1 or 2 using a nucleic acid polymers and nucleoside triphosphate substrates or their analogues and mixtures thereof.

5. The process of claim 1 or 2 wherein one primer is employed.

6. The process of claim 1 or 2 wherein the primer is from 9 to 10 nucleotides in length.

7. The process of claim 1 or 2 wherein at least one primer is biotinylated.

8. The process of claim 1 or 2 wherein the nucleic acids are from different individual organisms.

9. A process according to claim 4, wherein the nucleic acid polymerase is a DNA polymerase and the nucleoside triphosphate substrates are deoxyribonucleoside triphosphate substrates.

10. A process according to claim 9, wherein the DNA polymerase is a thermostable polymerase.

11. A process according to claim 10, wherein the DNA polymerase is Taq polymerase.

12. The process of claim 1 or 2, wherein the comparison is through size separation of the random extension products.

13. A process according to claim 12, wherein the size separation comparison is performed by electrophoresis through a polyacrylamide gel matrix or agarose gel matrix.

14. The process of claim 1 or 2, wherein the comparison is through nucleotide sequence determination.

15. The process of claim 1 or 2, wherein the nucleic acid source is selected from the group consisting of plants, animals and microbes.

16. The process of claim 1 or 2 wherein the nucleic acid source is human.

17. The process of claim 1 or 2 wherein a difference in the random extension products is used as a marker to construct a genetic map.

18. The process of claim 1 or 2 wherein a difference in the random extension products is used as a marker to distinguish or identify individuals.

* * * * *

-continued

tcctcctttt nntnnt 16

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We claim:

1. A binary composition for hybridizing to a target polynucleotide sequence comprising:

a probe comprising a target-specific portion and a clamp-specific portion wherein the target-specific portion is capable of sequence-specific binding to a target polynucleotide sequence; and

a clamp comprising two probe-specific portions, one or more labels and at least one nucleic acid analog selected from a nucleobase analog, a sugar analog, and an internucleotide analog;

wherein the probe is hybridized to the clamp by sequence-specific binding of the probe-specific portion of the clamp to the clamp-specific portion of the probe, and the clamp is incapable of sequence-specific binding to the target polynucleotide; and

wherein the two probe-specific portions are capable of sequence-specific binding to the clamp-specific portion of the probe to form a triplex.

2. The binary composition of claim 1 wherein the probe comprises 6 to 100 nucleotides.

3. The binary composition of claim 1 wherein the clamp-specific portion of the probe comprises purine nucleotides.

4. The binary composition of claim 1 wherein the probe-specific portion of the clamp comprises pyrimidine nucleobase analogs.

5. The binary composition of claim 1 wherein the clamp comprises 6 to 50 nucleic acid analogs.

6. The binary composition of claim 1 wherein the clamp sequence comprises $(CAG)_n$ where n=1–10.

7. The binary composition of claim 1 wherein the clamp sequences include $(TCC)_n$ and nucleic acid analogs which bind the probe sequence $(GGA)_n$ where n=1–10.

8. The binary composition of claim 1 wherein the probe comprises a nucleic acid analog selected from the group consisting of a nucleobase analog, a sugar analog, and an internucleotide analog.

9. The binary composition of claim 8 wherein the nucleobase analog is selected from the group consisting of C-5-

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We claim:

1. A method for detecting a target polynucleotide sequence comprising the steps of:

(a) amplifying a target polynucleotide with primer extension reagents in a first set of one or more vessels and amplifying an external control polynucleotide with the primer extension reagents in a second set of one or more vessels;

wherein the primer extension reagents include a forward primer, a reverse primer, one or more detectable probes, a polymerase, and one or more nucleotide 5'-triphosphates;

the forward primer and the detectable probe are separated by 0 to 5 nucleotides when hybridized to the external control polynucleotide, or its complement, and the reverse primer and the detectable probe are separated by 0 to 5 nucleotides when hybridized to the external control polynucleotide, or its complement; and

the external control polynucleotide begins the amplification process as a single-stranded polynucleotide; and

the external control polynucleotide is shorter than the target polynucleotide; and

(b) detecting a signal from said one or more detectable probes.

2. The method of claim 1 wherein said detectable probe is a self-quenching fluorescence probe comprising a reporter dye and a quencher.

3. The method of claim 2 wherein said polymerase cleaves said self-quenching fluorescence probes during amplification to separate said reporter dye from said quencher.

4. The method of claim 1 wherein the primer extension reagent of the second set of vessels comprises a first detectable probe and a second detectable probe; the sequence of the first probe differs from the second probe by one or more mismatches, insertions, or deletions, and the signal of the first probe is resolvable from the signal of the second probe.

5. The method of claim 2 wherein the primer extension reagent of the second set of vessels comprises a first self-quenching fluorescence probe and a second self-quenching fluorescence probe; the sequence of the first probe differs from the second probe by one or more mismatches, insertions, or deletions, and the reporter dye of the first probe is spectrally resolvable from the reporter dye of the second probe.

6. The method of claim 5 wherein the sequences of the first self-quenching fluorescence probe and second self-quenching fluorescence probe differ by a single nucleotide mismatch.

7. The method of claim 1 further comprising amplifying a second single-stranded external control polynucleotide with primer extension reagents in a third set of one or more vessels.

8. The method of claim 7 wherein the sequence of the first single-stranded external control polynucleotide differs from the second single-stranded external control polynucleotide by one or more mismatches, insertions, or deletions.

9. The method of claim 7 wherein the sequence portion of the first single-stranded external control polynucleotide complementary to a detectable probe differs by a single nucleotide from the sequence portion of the second single-stranded external control polynucleotide complementary to a detectable probe.

10. The method of claim 7 further comprising a fourth set of one or more vessels including primer extension reagents.

11. The method of claim 1 wherein the target polynucleotide is selected from the group consisting of a plasmid, a cDNA, a PCR product, genomic DNA, a restriction digest, and a ligation product.

12. The method of claim 1 wherein the forward primer or the reverse primer, and the detectable probe are adjacent when hybridized to the single-stranded external control polynucleotide, or its complement.

13. The method of claim 1 wherein the external control polynucleotide, or its complement, forms single-stranded overhangs consisting of 0 to about 10 nucleotides when hybridized to the forward primer or to the reverse primer.

14. The method of claim 1 wherein the forward primer and reverse primer are each 10 to 40 nucleotides in length.

15. The method of claim 2 wherein the self-quenching fluorescence probe is 10 to 40 nucleotides in length.

16. The method of claim 1 wherein the single-stranded external control polynucleotide is 30 to 110 nucleotides in length.

17. The method of claim 1 wherein the single-stranded external control polynucleotide is 50 to 70 nucleotides in length.

18. The method of claim 1 wherein a change in fluorescence intensity is detected as an indication of the presence of the target sequence.

19. The method of claim 1 wherein the products of the nucleic acid amplification of the target polynucleotide and the external control polynucleotide are detected by end-point analysis.

20. The method of claim 1 wherein the products of the nucleic acid amplification of the target polynucleotide and the external control polynucleotide are detected during real-time analysis.

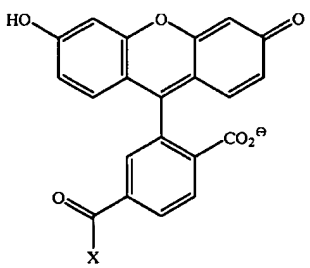
21. The method of claim 1 wherein the products of the nucleic acid amplification of the target polynucleotide and the external control polynucleotide are detected by fluorescence.

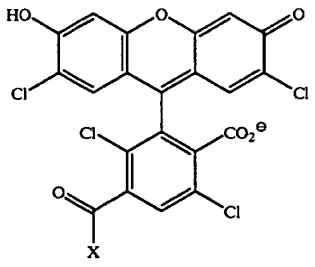
22. The method of claim 1 wherein said nucleic acid polymerase is a thermostable polymerase with 5' nuclease activity.

23. The method of claim 2 wherein said reporter is a xanthene dye.

24. The method of claim 23 wherein said xanthene dye is a fluorescein dye.

25. The method of claim 24 wherein said fluorescein dye is selected from the group consisting of:





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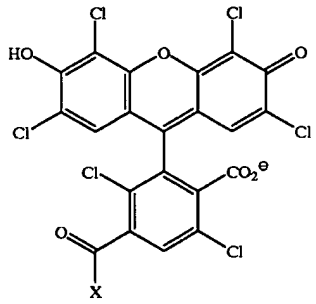
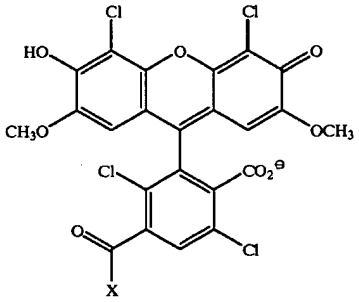
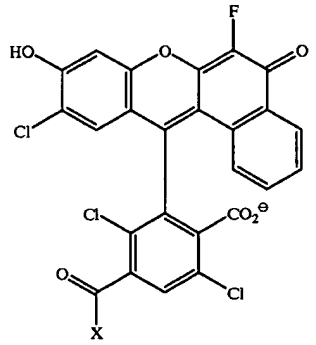
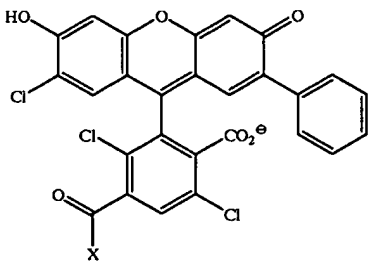


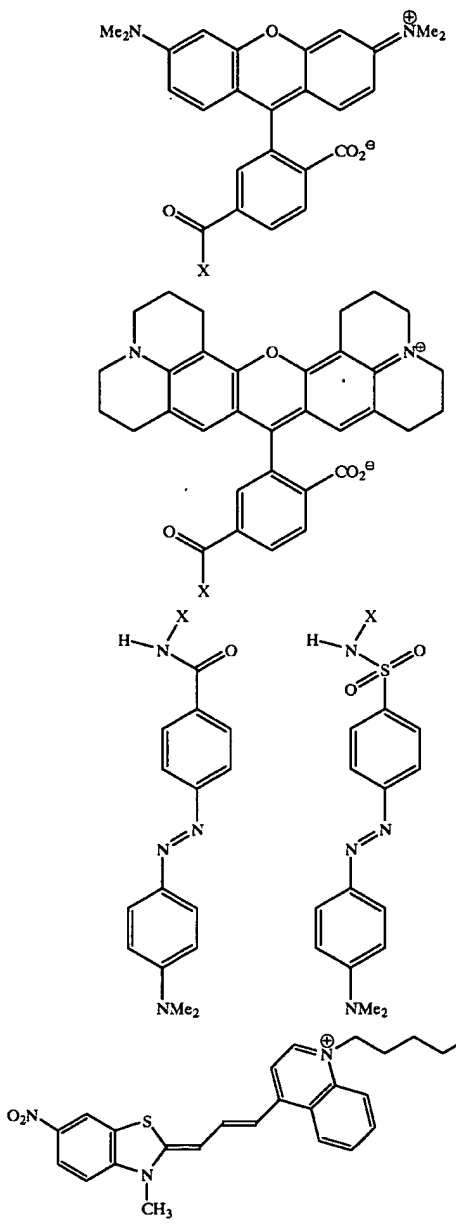






where X is an attached site to the probe.

26. The method of claim 2 wherein said quencher is selected from the group consisting of:



where X is an attachment site to the probe.

27. The method of claim 2 wherein said reporter dye is separated from said quencher by at least 12 nucleotides.

28. The method of claim 2 wherein said reporter dye is attached at the 5' terminus or 3' terminus of the self-quenching fluorescence probe.

29. The method of claim 2 wherein said quencher is attached at the 5' terminus or 3' terminus of the self-quenching fluorescence probe.

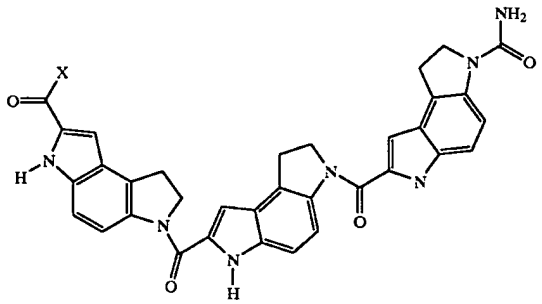
30. The method of claim 2 wherein said quencher is non-fluorescent.

31. The method of claim 1 wherein the detectable probe is labelled with a minor groove binder.

32. The method of claim 2 wherein the self-quenching fluorescence probe is labelled with a minor groove binder.

33. The method of claim 32 wherein the self-quenching fluorescence probe is labelled with a minor groove binder at the 3' tenninus nucleotide.

34. The method of claim 32 wherein the minor groove binder has the structure:



where X is an attachment site to the probe.

35. The method of claim 1 where one or more nucleotide 5'-triphosphates are labelled.

36. The method of claim 35 wherein the label is a fluorescent dye, a quencher, biotin, or a minor groove binder.

37. The method of claim 1 wherein the vessels are located in a microwell tray.

38. The method of claim 1 wherein the primer extension reagents are delivered to the vessels by robotic means.

39. The method of claim 1 wherein amplification is conducted by a thermal cycler.

* * * * *

-continued

(A) LENGTH: 21 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CAAGCTTCCC GTTCTCAGCC T 21

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ACCGTCAAGG CTGAGAACGG GAAGCTTGTC 30

What is claimed is:

1. A method for detecting a target polynucleotide in a sample comprising:

contacting said sample of nucleic acids with an oligonucleotide probe under conditions where said oligonucleotide probe selectively hybridizes to said target polynucleotide, said oligonucleotide probe including a fluorescent reporter molecule and a quencher molecule capable of quenching the fluorescence of said reporter molecule which are attached to said oligonucleotide probe such that said oligonucleotide probe is capable of adopting at least one single-stranded conformation when not hybridized to said target polynucleotide where said quencher molecule quenches the fluorescence of said reporter molecule and is capable of adopting at least one conformation when hybridized to said target polynucleotide where the fluorescence of said reporter molecule is unquenched such that the fluorescence intensity of said reporter molecule when said oligonucleotide probe is hybridized to said target polynucleotide is greater than the fluorescence intensity of said reporter molecule when said oligonucleotide probe is not hybridized to said target polynucleotide and said oligonucleotide probe is not hybridized with itself in the form of a hairpin structure; and

monitoring the fluorescence of said reporter molecule fluorescence intensity of said reporter molecule indicating the presence of said under conditions where said oligonucleotide probe does not hybridize with itself to form a hairpin structure in order to detect the hybridization of said target polynucleotide to said oligonucleotide probe.

2. The method according to claim 1 wherein the fluorescence intensity of said reporter molecule under conditions where said oligonucleotide probe does not hybridize with itself to form a hairpin structure is at least about a factor of 6 greater when said oligonucleotide probe is hybridized to said target polynucleotide than when said oligonucleotide probe is not hybridized to said target polynucleotide.

3. The method according to claim 1 wherein said reporter molecule is separated from said quencher molecule by at least 15 nucleotides.

4. The method according to claim 1 wherein said reporter molecule is separated from said quencher molecule by between 15 and 60 nucleotides.

5. The method according to claim 1 wherein said reporter molecule is separated from said quencher molecule by at least 18 nucleotides.

6. The method according to claim 5 wherein said reporter molecule is separated from said quencher molecule by between 18 and 30 nucleotides.

7. The method according to claim 1 wherein said reporter molecule is attached to a 3' terminal nucleotide of said oligonucleotide probe.

8. The method according to claim 7 wherein said quencher molecule is attached to a 5' terminal nucleotide of said oligonucleotide probe.

9. The method according to claim 1 wherein said reporter molecule is attached to a 5' terminal nucleotide of said oligonucleotide probe.

10. The method according to claim 9 wherein said quencher molecule is attached to a 3' terminal nucleotide of said oligonucleotide probe.

11. The method according to claim 1 wherein said quencher molecule is attached to a 3' terminal nucleotide of said oligonucleotide probe.

12. The method according to claim 1 wherein said quencher molecule is attached to a 5' terminal nucleotide of said oligonucleotide probe.

13. The method according to claim 1 wherein said reporter molecule is a fluorescein dye and said quencher molecule is a rhodamine dye.

14. The method according to claim 1 wherein said quencher is fluorescent and the fluorescence intensity of said reporter molecule is greater than the fluorescence intensity of said quencher molecule when said oligonucleotide probe is hybridized to said target polynucleotide.

15. The method according to claim 14 wherein the fluorescence intensity of said reporter molecule is at least about a factor of 3.5 greater than the fluorescence intensity of said quencher molecule when said probe is hybridized to said target polynucleotide.

16. A method for detecting a target polynucleotide in a sample comprising:

contacting said sample of nucleic acids with an oligonucleotide probe under conditions where said oligonucleotide probe selectively hybridizes to said target polynucleotide, said oligonucleotide probe including a fluorescent reporter molecule and a fluorescent quencher molecule capable of quenching the fluorescence of said reporter molecule which are attached to said oligonucleotide probe such that said oligonucleotide probe is capable of adopting at least one single-stranded conformation when not hybridized to said

target polynucleotide where said fluorescent quencher molecule quenches the fluorescence of said reporter molecule and is capable of adopting a least one conformation when hybridized to said target polynucleotide where the fluorescence of said reporter molecule is unquenched such that the ratio of the fluorescence intensities of said reporter molecule to said fluorescent quencher molecule when said oligonucleotide sequence is hybridized to said target polynucleotide is greater than the ratio of the fluorescence intensities of said reporter molecule to said fluorescent quencher molecule when said oligonucleotide probe is not hybridized to said target polynucleotide and said oligonucleotide probe is not hybridized with itself in the form of a hairpin structure; and

monitoring the ratio between the fluorescence of said reporter molecule and said fluorescent quencher molecule under conditions where said oligonucleotide probe does not hybridize with itself to form a hairpin structure in order to detect the hybridization of said target polynucleotide to said oligonucleotide probe.

17. The method according to claim **16** wherein the ratio of the fluorescence intensities of said reporter molecule to said quencher molecule when said oligonucleotide probe is hybridized to said target polynucleotide is at least a factor of 6 greater than the ratio of the fluorescence intensities of said reporter molecule to said quencher molecule when said oligonucleotide probe is not hybridized to said target polynucleotide.

18. A method for detecting a target polynucleotide in a sample comprising:

contacting a sample of nucleic acids with an oligonucleotide probe attached to a solid support under conditions favorable for hybridization of said oligonucleotide probe to said target polynucleotide, said oligonucleotide probe including a fluorescent reporter molecule and a quencher molecule capable of quenching the fluorescence of said reporter molecule which are attached to said oligonucleotide probe such that said oligonucleotide probe is capable of adopting at least one single-stranded conformation when not hybridized to said target polynucleotide where said quencher molecule quenches the fluorescence of said reporter molecule and is capable of adopting at least one conformation when hybridized to said target polynucleotide where the fluorescence of said reporter molecule is unquenched such that the fluorescence intensity of said reporter molecule when said oligonucleotide probe is hybridized to said target polynucleotide is greater than the fluorescence intensity of said reporter molecule when said oligonucleotide probe is not hybridized to said target polynucleotide and said oligonucleotide probe is not hybridized with itself in the form of a hairpin structure; and

monitoring the fluorescence of said reporter molecule under conditions where said oligonucleotide probe does not hybridize with itself to form a hairpin structure in order to detect the hybridization of said target polynucleotide to said oligonucleotide probe.

19. The method according to claim **18** wherein the fluorescence intensity of said reporter molecule under conditions where said oligonucleotide probe does not hybridize with itself to form a hairpin structure is at least about a factor of 6 greater when said oligonucleotide probe is hybridized to said target polynucleotide than when said oligonucleotide probe is not hybridized to said target polynucleotide.

20. The method according to claim **18** wherein said reporter molecule is separated from said quencher molecule by at least 15 nucleotides.

21. The method according to claim **20** wherein said reporter molecule is separated from said quencher molecule by between 15 and 60 nucleotides.

22. The method according to claim **18** wherein said reporter molecule is separated from said quencher molecule by at least 18 nucleotides.

23. The method according to claim **22** wherein said reporter molecule is separated from said quencher molecule by between 18 and 30 nucleotides.

24. The method according to claim **18** wherein said reporter molecule is attached to a 3' terminal nucleotide of said oligonucleotide probe.

25. The method according to claim **24** wherein said quencher molecule is attached to a 5' terminal nucleotide of said oligonucleotide probe.

26. The method according to claim **18** wherein said reporter molecule is attached to a 5' terminal nucleotide of said oligonucleotide probe.

27. The method according to claim **26** wherein said quencher molecule is attached to a 3' terminal nucleotide of said oligonucleotide probe.

28. The method according to claim **18** wherein said quencher molecule is attached to a 3' terminal nucleotide of said oligonucleotide probe.

29. The method according to claim **18** wherein said quencher molecule is attached to a 5' terminal nucleotide of said oligonucleotide probe.

30. The method according to claim **18** wherein said reporter molecule is a fluorescein dye and said quencher molecule is a rhodamine dye.

31. The method according to claim **18** wherein said probe is attached to said solid support by a linker.

32. The method according to claim **31** wherein said linker separates said probe from said solid support by at least 30 atoms.

33. The method according to claim **32** wherein said linker separates said probe from said solid support by at least 50 atoms.

34. The method according to claim **31** wherein said linker is a functionalized polyethylene glycol.

35. The method according to claim **34** wherein said linker is a polynucleotide.

36. The method according to claim **18** wherein said quencher molecule is fluorescent and the fluorescence intensity of said reporter molecule is greater than the fluorescence intensity of said quencher molecule when said oligonucleotide probe is hybridized to said target polynucleotide.

37. The method according to claim **36** wherein the fluorescence intensity of said reporter molecule is at least about a factor of 3.5 greater than the fluorescence intensity of said quencher molecule when said probe is hybridized to said target polynucleotide.

38. A method for detecting a target polynucleotide in a sample comprising:

contacting said sample of nucleic acids with an oligonucleotide probe attached to a solid support under conditions where said oligonucleotide probe selectively hybridizes to said target polynucleotide, said oligonucleotide probe including a fluorescent reporter molecule and a fluorescent quencher molecule capable of quenching the fluorescence of said reporter molecule which are attached to said oligonucleotide probe such that said oligonucleotide probe is capable of adopting at least one single-stranded conformation when not hybridized to said target polynucleotide where said fluorescent quencher molecule quenches the fluorescence of said reporter molecule and is capable of

adopting at least one conformation when hybridized to said target polynucleotide where the fluorescence of said reporter molecule is unquenched such that the ratio of the fluorescence intensities of said reporter molecule to said fluorescent quencher molecule when said oligonucleotide sequence is hybridized to said target polynucleotide is greater than the ratio of the fluorescence intensities of said reporter molecule to said fluorescent quencher molecule when said oligonucleotide probe is not hybridized to said target polynucleotide and said oligonucleotide probe is not hybridized with itself in the form of a hairpin structure; and

monitoring the ratio between the fluorescence of said reporter molecule and said fluorescent quencher molecule under conditions where said oligonucleotide probe does not hybridize with itself to form a hairpin structure in order to detect the hybridization of said target polynucleotide to said oligonucleotide probe.

39. The method according to claim **38** wherein the ratio of the fluorescence intensities of said reporter molecule to said quencher molecule when said oligonucleotide sequence is hybridized to said target polynucleotide is at least a factor of 6 greater than the ratio of the fluorescence intensities of said reporter molecule to said quencher molecule when said oligonucleotide sequence is not hybridized to said target polynucleotide.

* * * * *

C) Piperidine Cleavage

Chemical cleavage of the C and T bases that react with hydroxylamine or osmium tetroxide is achieved by incubating the dishes with 1M piperidine at 90° C. for 30 min. The wells are then washed extensively with distilled water.

EXAMPLE 5

Sequencing of Mismatch Regions

Immobilized DNAs prepared as described in Examples 1 and 2 above and subjected to mismatch recognition and cleavage (as described in Examples 3 or 4 above or by other methods) are incubated with a single-stranded oligonucleotide having the sequence 5'-CAGTAGTACAACTGACCCTTTTGGGACCGC-3' [SEQ ID NO:1] under conditions in which efficient ligation of the oligonucleotide to free 5' ends is achieved. The oligonucleotide and immobilized DNA are combined in a solution containing 50 mM Tris HCl, pH 7.5, 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP, and 100 μg/ml bovine serum albumin, after which RNA ligase (Pharmacia Biotech, Uppsala, Sweden) is added to the solution to achieve a final enzyme concentration of 0.1–5.0 U/ml. The reaction is allowed to proceed at 37° C. for 15 min. Following the ligation reaction, the solution is removed, and the wells are washed with distilled water.

DNA sequencing is then performed using the Sanger method (Sanger et al., *Proc. Natl. Acad. Sci. U.S.A.* 74:5463, 1977).

EXAMPLE 6

Positional Cloning of a Disease-causing Gene

The experiments described below are performed to rapidly localize and sequence a genomic region corresponding to a disease-causing gene.

A multiplex family in which a genetic disease is expressed is identified using standard clinical indicators. DNA samples are obtained from affected and unaffected individuals as described in Example 1 above; if by patterns of transmission the disease appears to be an autosomal recessive syndrome, DNA samples are obtained from those individuals presumptively heterozygous for the disease gene.

All DNA samples are subjected to mismatch analysis by hybridization to wild-type DNA as described in Example 2 above. The hybrids are then treated with T4 endonuclease 7 as described in Example 3 above. Finally, an oligonucleotide having the sequence 5'-CAGTAGTACAACTGACCCTTTTGGGACCGC-3' [SEQ ID NO:1] is ligated to the cleaved hybrids using RNA ligase, and the products are subjected to enzymatic DNA sequencing as described in Example 5 above.

The sequences obtained from unaffected, affected, and presumptively heterozygous family members are compared with each other and with available sequence databases, using, for example, Sequencher (Gene Codes, Ann Arbor, Mich.) and Assembly Lign (Kodak, New Haven, Conn.) The sequences are also serve as the basis for design of oligonucleotide probes, which are chemically synthesized and used to probe human genomic DNA libraries.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 1

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc ="synthetic oligonucleotide"

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGTAGTACA ACTGACCCTT TTGGGACCGC 30

What is claimed is:

1. A method for identifying one or more genetic alterations in a target sequence present in a DNA sample, which comprises:

a) immobilizing a first DNA sample, said first DNA having a 5' end and a 3' end on a solid support under conditions such that said 5' end is bound to said support and said 3' end is unbound;

b) hybridizing said immobilized sample with a second DNA having a 5' end and a 3' end wherein said second DNA does not contain the alteration(s), to form heteroduplex DNA containing a mismatch region at the site of an alteration(s);

c) cleaving one or both strands of said heteroduplex adjacent to said mismatch region to form a gap at or in the vicinity of said alteration;

d) subjecting said cleaved heteroduplex to conditions of denaturation to dissociate said second DNA and cleaved first DNA 3' to the site of cleavage from immobilized remaining first DNA;

e) removing DNA strands cleaved in step c and dissociated in step d from said immobilized remaining first DNA;

f) ligating a single-stranded oligonucleotide primer of known sequence to the unbound end of said immobilized remaining first DNA to form a ligation product;

(g) treating said ligation product with a DNA polymerase and an oligonucleotide complementary to said primer of known sequence in the presence of dideoxynucleotides or four nucleotide triphosphates and determining the nucleotide sequence adjacent to the ligated primer sequence; and

h) comparing said nucleotide sequence with a predetermined cognate wild-type sequence to identify said genetic alteration(s).

2. The method of claim 1, wherein the alterations are selected from the group consisting of additions, deletions, and substitutions of one or more nucleotides and combinations thereof.

3. The method of claim 1 further comprising blocking unbound ends on said heteroduplex DNA prior to the cleaving step.

4. The method of claim 3, wherein the blocking step comprises a method selected from the group consisting of removal of 5' phosphate groups, homopolymeric tailing of 3' ends with dideoxynucleotides, and ligation of modified double-stranded oligonucleotides.

5. The method of claim 1, wherein said target sequence is amplified prior to the immobilizing step.

6. The method of claim 1, wherein the solid support is selected from the group consisting of nitrocellulose filter, nylon filter, glass beads, and plastic.

7. The method of claim 1, wherein said cleaving step comprises exposing said heteroduplex DNA to one or more mismatch repair proteins under conditions appropriate for mismatch recognition and cleavage.

8. The method of claim 7, wherein the one or more mismatch repair proteins are selected from the group consisting of *Eschericia coli* proteins MutY, MutS, MutL, MutH, and combinations thereof, or functional homologues thereof.

9. The method of claim 8, wherein the functional homologues are derived from species selected from the group consisting of *Salmonella typhimurium, Streptococcus pneumoniae, Saccharomyces cerevisiae, Schizosaccharomyces pombe,* mouse and human.

10. The method of claim 1, wherein said cleaving step comprises exposing said heteroduplex DNA to a mixture of nucleotide excision repair proteins under conditions appropriate for mismatch recognition and cleavage.

11. The method of claim 10, wherein the mixture comprises *E. coli* proteins UvrA, UvrB, and UvrC, or functional homologues thereof.

12. The method of claim 11, wherein the functional homologues are derived from species selected from the group consisting of *Saccharomyces cerevisiae* and human.

13. The method of claim 1, wherein said cleaving step comprises exposing said heteroduplex DNA to one or more resolvase proteins under conditions appropriate for mismatch recognition and cleavage.

14. The method of claim 13, wherein the resolvases are selected from the group consisting of bacteriophage T4 Endonuclease VII and bacteriophage T7 endonuclease I.

15. The method of claim 1, wherein said cleaving step comprises the steps of:

(i) exposing said heteroduplex DNA to one or more chemical reagents under conditions appropriate for mismatch recognition and modification; and

(ii) contacting recognized and modified heteroduplex DNA with one or more reagents to selectively cleave one strand in the vicinity of the modification.

16. The method of claim 15, wherein the chemical reagent is selected from the group consisting of hydroxylamine and osmium tetroxide.

17. The method of claim 1, wherein the single-stranded oligonucleotide primer is from about 15 to about 35 nucleotides.

18. The method of claim 1, wherein the ligating step is performed in the presence of RNA ligase.

19. The method of claim 1, wherein the determining step is performed by enzymatic DNA sequencing.

20. The method of claim 1, wherein the determining step is performed by hybridization to oligonucleotide arrays.

21. A method for identifying one or more genetic alterations in a target sequence present in a genomic DNA sample, which comprises:

a) immobilizing a first DNA sample, said first DNA having a 5' end and a 3' end on a solid support under conditions such that said 5' end is bound to said support and said 3' end is unbound;

b) hybridizing said immobilized DNA sample with a second DNA sample, said second DNA having a 5' end and a 3' end, and wherein said second sample does not contain the alteration(s), to form heteroduplex DNA containing a mismatch region at the site of an alteration (s);

c) treating said heteroduplex with terminal transferase in the presence of a dideoxynucleotide to block unbound ends thereof;

d) contacting said heteroduplex with bacteriophage T4 endonuclease 7 to cleave one or both strands of said heteroduplex adjacent to said mismatch region to form a gap at or in the vicinity of said alteration;

e) subjecting said cleaved heteroduplex to conditions of denaturation to dissociate said second DNA and cleaved first DNA 3' to the site of cleavage from immobilized remaining first DNA;

f) removing DNA strands cleaved in step d and dissociated in step (e) from said immobilized remaining first DNA;

g) ligating a single-stranded oligonucleotide primer having the sequence 5'-CAGTAGTACAACTGACCCTTTTGGGACCGC-3' (SEQ ID NO:1) to the unbound end of immobilized remaining first DNA to form a ligation product;

(h) treating said ligation product with a DNA polymerase and an oligonucleotide complementary to said primer of known sequence in the presence of dideoxynucleotides or four nucleotide triphosphates and determining the nucleotide sequence adjacent to ligated primer sequence; and

i) comparing said nucleotide sequence with a predetermined cognate wild-type sequence to identify said alteration(s).

22. A method for identification of one or more mutation(s) in a DNA, which comprises:

a) immobilizing one or more first DNA samples, said first DNA having a respective 5' end and a respective 3' end, on a solid support under conditions such that said 5' end is bound to said support and said 3' and is unbound;

b) hybridizing said immobilized sample(s) with a second DNA sample, said second DNA having a respective 5' end and a respective 3' end, and wherein said second

sample does not contain the mutation(s), to form heteroduplex DNA containing a mismatch region at the site of a mutation;

c) chemically blocking unbound ends on said heteroduplex DNA;

d) treating said heteroduplex DNA so that one or both strands are cleaved within or adjacent to said mismatch region to form a gap at or in the vicinity of said alteration;

e) subjecting said cleaved heteroduplex to conditions of denaturation to dissociate said second DNA and cleaved first DNA 3' to the site of cleavage from immobilized remaining first DNA;

f) removing DNA strands cleaved in step (d) and dissociated in step (e) from immobilized remaining first DNA;

g) ligating a single-stranded oligonucleotide primer of known sequence to the unbound end of said immobilized remaining first DNA to form a ligation product;

(h) treating said ligation product with a DNA polymerase and an oligonucleotide complementary to said primer of known sequence in the presence of dideoxynucleotides or four nucleotide triphosphates and determining the nucleotide sequence adjacent to ligated primer sequence; and

i) comparing said nucleotide sequence with one or more predetermined cognate wild-type sequences to identify said mutation(s).

23. A method for multiplex identification of one or more mutations in a DNA, the method comprising:

a) obtaining one or more first DNA samples, said DNA having a respective 5' end and a respective 3' end;

b) amplifying one or more target sequences in each of said samples;

c) immobilizing said amplified sequences on a solid support under conditions such that said 5' end is bound to said support and said 3' end is unbound;

d) hybridizing said immobilized sample(s) with a second DNA sample, said second DNA having a respective 5' end and a respective 3' end, and wherein said second sample does not contain the mutation(s), to form heteroduplex DNA containing a mismatch region at the site of a mutation;

e) chemically blocking unbound ends on said heteroduplex DNA;

f) treating said heteroduplex DNA so that one or both strands are cleaved within or adjacent to said mismatch region to form a gap at or in the vicinity of said mutation;

g) subjecting said cleaved heteroduplex to conditions of denaturation to dissociate said second DNA and cleaved first DNA 3' to site of cleavage from immobilized remaining first DNA;

h) removing DNA strands cleaved in step (f) and dissociated in step (g) from said immobilized remaining first DNA;

i) ligating a single-stranded oligonucleotide primer of known sequence to the unbound end of said immobilized remaining first DNA to form a ligation product;

(j) treating said ligation product with a DNA polymerase and an oligonucleotide complementary to said primer of known sequence in the presence of dideoxynucleotides or four nucleotide triphosphates and determining the nucleotide sequence adjacent to ligated primer sequence; and

k) comparing said nucleotide sequence with one or more predetermined cognate wild-type sequences to identify said mutation(s).

24. The method of claim **1** wherein the DNA samples are denatured prior to hybridization.

25. The method of claim **21** wherein the DNA samples are denatured prior to hybridization.

26. The method of claim **22** wherein the DNA samples are denatured prior to hybridization.

27. The method of claim **23** wherein the DNA samples are denatured prior to hybridization.

28. A method for positional cloning of a gene of interest, the method comprising:

a) immobilizing a first DNA sample from an individual displaying a given phenotype, said first DNA having a 5' end and a 3' end on a solid support under conditions such that said 5' end is bound to said support and said 3' end unbound;

b) hybridizing said immobilized sample with a second DNA sample, said second DNA having a 5' end and a 3' end, and wherein said second DNA sample is from one or more individual(s) not displaying said phenotype to form heteroduplex DNA containing a mismatch region at the site of a genetic alteration;

c) cleaving one or both strands of said heteroduplex DNA to form a gap at or in the vicinity of said alteration;

d) subjecting said cleaved heteroduplex to conditions of denaturation to dissociate said second DNA from said first DNA;

e) removing DNA strands cleaved in step (c) and dissociated in step (d) from said first DNA;

f) ligating a single-stranded oligonucleotide primer of known sequence to the 3' end of first cleaved DNA to form a ligation product;

g) treating said ligation product with a DNA polymerase and an oligonucleotide complementary to said primer of known sequence in the presence of dideoxynucleotides or four nucleotide triphosphates and determining the nucleotide sequence adjacent to ligated primer sequence;

h) preparing a synthetic oligonucleotide comprising all or part of said determined nucleotide sequence; and

i) identifying a DNA clone that hybridizes to said oligonucleotide.

29. The method of claim **28** which comprises chemically blocking free terminal ends of said heteroduplex prior to said cleaving steps.

30. The method of claim **28**, wherein the genetic alterations are selected from the group consisting of additions, deletions, and substitutions of one or more nucleotides and combinations thereof.

31. The method of claim **28**, wherein the cleaving step comprises exposing the heteroduplex DNA to a mixture of mismatch repair proteins under conditions appropriate for mismatch recognition and cleavage.

32. The method of claim **31**, wherein the mismatch repair proteins are selected from the group consisting of *Escherichia coli* proteins MutY, MutS, MutL, MutH, and combinations thereof, or functional homologues thereof.

33. The method of claim **32**, wherein the functional homologues are from species selected from the group consisting of *Salmonella typhimurium, Streptococcus pneumoniae, Saccharomyces cerevisiae, Schizosaccharomyces pombe,* mouse and human.

34. The method of claim **28**, wherein the cleaving step comprises exposing the heteroduplex DNA to a mixture of

nucleotide excision repair proteins under conditions appropriate for mismatch recognition and cleavage.

35. The method of claim 34, wherein the mixture comprises *Escherichia coli* proteins UvrA, UvrB, and UvrC, or functional homologues thereof.

36. The method of claim 35, wherein the functional homologues are from species selected from the group consisting of *Saccharomyces cerevisiae* and human.

37. The method of claim 28, wherein the cleaving step comprises exposing the heteroduplex DNA to one or more resolvase proteins under conditions appropriate for mismatch recognition and cleavage.

38. The method of claim 37, wherein the resolvases are selected from the group consisting of bacteriophage T4 Endonuclease VII and bacteriophage T7 endonuclease I.

39. The method of claim 28, wherein the cleaving step comprises the steps of:

(i) exposing the heteroduplex to one or more chemical reagents under conditions appropriate for mismatch recognition and modification; and

(ii) contacting said recognized and modified heteroduplex DNA with one or more reagents to selectively cleave one strand in the vicinity of the alteration.

40. The method of claim 39, wherein the chemical reagent is selected from the group consisting of hydroxylamine and osmium tetroxide.

41. The method of claim 28, wherein the single-stranded oligonucleotide primer is from about 15 to about 35 nucleotides.

42. The method of claim 28, wherein the ligating step is performed in the presence of RNA ligase.

43. The method of claim 28, wherein the determining step performed by enzymatic DNA sequencing.

44. The method of claim 28, wherein the determining step is performed by hybridization to oligonucleotide arrays.

45. The method of claim 28, wherein the identifying step is achieved using a method selected from the group consisting of colony hybridization, identification of tissue-specific expression, reverse transcription-amplification of mRNA, and screening of an affected population for genotype/phenotype association.

46. The method of claim 28, wherein the DNA samples are denatured prior to hybridization.

47. The method of claim 28, wherein the first DNA is immobilized on a solid support under conditions such that said 5' end is bound to said support and said 3' end is unbound.

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